IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Dahl *et al.*		
Serial No.:	10/719,913	Group No.:	1637
Filed:	11/21/03	Examiner:	Staples
Entitled:	**Methods for Making Transcription Products**		

DECLARATION OF INVENTOR GARY A. DAHL, PhD UNDER 37 CFR § 1.132

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

To Whom It May Concern:

I, Gary A. Dahl, hereby declare and state, under penalty of perjury, that:

1. I am President and CEO of EPICENTRE Technologies Corporation in Madison Wisconsin, which I co-founded more that 22 years ago with Dr. Jerry Jendrisak, the Vice President of Research and Development and a co-inventor of the present patent application. I received a Ph.D. from the McArdle Laboratory for Cancer Research at the University of Wisconsin, Madison, and did more than three years of postdoctoral research at the University of Paris-South, Orsay, France before beginning work in the biotechnology industry. Dr. Jendrisak also received a Ph.D. from the University of Wisconsin, Madison and did post-doctoral work at the McArdle Laboratory. Both Dr. Jendrisak and I have many years of experience working with RNA polymerases, and *in vitro* transcription and RNA amplification methods, which experience pre-dates our founding of EPICENTRE. For example, Dr. Jendrisak was the first person to isolate wheat germ RNA polymerase as a Ph.D. student in the early

1970's and the methods he developed to purify *E. coli* RNA polymerase holoenzyme and core enzyme as a post-doctoral fellow in the mid-1970's are still the preferred methods used today. After his postdoctoral work, he continued to focus his research on RNA polymerases and *in vitro* transcription as a Professor at the University of Minnesota-Minneapolis, at which institution he was rapidly appointed to a Full Professorship. From that position, he was recruited to the position of Director of Research at Promega Corporation, where he oversaw the launch of RNasin™ RNase inhibitor and the RiboProbe™ *In Vitro* Transcription Systems, which became important products for the company and made significant contributions to research by providing researchers new capabilities to produce and work with RNA *in vitro*. While at Promega, he was also an inventor of the world's first dual-promoter vectors (U.S. Patent No. 4,766,072), which are widely used to this day. I worked with Dr. Jendrisak at Promega, from 1985 until the end of 1987, when we founded EPICENTRE. At EPICENTRE, we continued to focus much of our efforts on development of new RNA and *in vitro* transcription products. I believe we were the first to clone SP6 RNA polymerase, and we were the first company to offer very high concentrations of phage T7, T3 and SP6 RNA polymerases. Our high-concentration T7 RNA polymerase enzyme was used by Dr. Jim Eberwine and his associates for the so-called "Eberwine aRNA amplification method," which they developed, first at Stanford, and later at the University of Pennsylvania, for making labeled anti-sense target RNA (aRNA) from minute quantities of mRNA, especially for gene expression analysis using microarrays. EPICENTRE's TargetAmp™ aminoallyl aRNA amplification kit is the most sensitive Eberwine-type aRNA amplification kit available, permitting generation of labeled target RNA for microarray analysis from a single cell. We have also focused on developing and commercializing a wide variety of other products for RNA research, *in vitro* transcription, and RNA amplification. For example, we also sell the RiboMultiplier™ sense RNA amplification kit. We have filed numerous patent applications (including many of which I am an inventor) for new technologies in the areas of *in vitro* transcription and RNA amplification.

2. I am one of the inventors of the above-named patent application (hereinafter "present application").

3. I understand that certain claims of the present application are directed toward methods for making transcription products corresponding to target nucleic acid sequences in a sample by: extension of a sense promoter primer using a DNA polymerase; ligation of sense promoter-containing first-strand cDNA to form circular sense promoter-containing first-strand cDNA, which is a covalently-closed circle; annealing of an anti-sense promoter oligonucleotide to the sense promoter sequence; and transcription of the resulting transcription substrate. In some embodiments of the method, the circular transcription substrate is linearized to generate a linear transcription substrate. In some other embodiments, the circular sense promoter-containing first-strand cDNA is linearized to generate linear sense promoter-containing first-strand cDNA (which exhibits the sense promoter sequence on its 3'-end), and then the anti-sense promoter oligonucleotide is annealed to the sense promoter sequence to generate a linear transcription substrate. Thus, the invention also includes methods wherein the transcription products are generated from linear transcription substrates rather than from circular transcription substrates.

4. I believe, based on my experience in the field, that the present claims employ a new and novel composition - *a sense promoter primer* - that was not previously known or used in the art. I further believe that the present claims provide new and novel methods for using the sense promoter primer in order to amplify target nucleic acid sequences exhibited by one or multiple target nucleic acids in a sample. Some of the various embodiments of the methods use a sense promoter primer to yield RNA products that exhibit sequences which are identical to target nucleic acid sequences in the sample.

5. A "sense promoter primer" is an important and I believe a novel composition, the nature of which is described and defined in the present application. For example, paragraph [0185] of the present application defines a promoter primer, a sense promoter primer and an anti-sense promoter primer as follows:

> [0185] *A "promoter primer" is a primer, generally with a free 3'-OH group, that comprises a sequence that is complementary to a target sequence at its 3'-end and which encodes a transcription promoter in its 5'-portion. The transcription promoter in the 5'-end portion can*

be either a "sense" promoter" or an "anti-sense promoter." As defined herein, the promoter sequence of a double-stranded promoter that is operably joined to the 3'-end of the template strand sequence that is transcribed is a "sense promoter sequence" and a promoter primer that comprises this sequence is "a sense promoter primer." The sequence of a double-stranded promoter that is complementary to the sense promoter is defined herein as "an anti-sense promoter" and a promoter primer that comprises this sequence is "an anti-sense promoter primer."

Thus, a sense promoter primer is not merely an oligonucleotide or a polynucleotide, but is an oligonucleotide that is used as a primer. Further, a sense promoter primer exhibits a sense promoter sequence in its 5'-portion. Prior to the present application, I believe a sense promoter primer composition was unknown in the art and had never been used in a method to amplify one or multiple target nucleic acid sequences.

6. A sense promoter primer is a defined term (in the present application) and an oligonucleotide used as a sense promoter primer in a method of the present claims must comply with the definition in the present application. As such, a sense promoter primer must exhibit a sense promoter sequence in its 5'-portion. Specific examples of sequences exhibited by a sense promoter and an anti-sense promoter are given for a T7 RNA polymerase promoter in paragraphs [0013]-[0016] - at the beginning of the Brief Summary of the Invention – in order to emphasize the distinction. A sense promoter primer is not a primer that synthesizes sense RNA; it is a primer that exhibits a sense promoter sequence in its 5'-portion. Methods exist in the prior art that synthesize sense RNA, but those prior art references did not use a sense promoter primer to do so.

7. I believe that one important reason the claimed method is new and not obvious from the methods previously known in the art is because it shows how to use a sense promoter primer to amplify a target nucleic acid sequence to which the 3'-portion of the sense promoter primer anneals and is extended by a DNA polymerase, including an RNA-dependent DNA polymerase or reverse transcriptase.

8. In order to emphasize the importance of the sense promoter primer, reference is made to Figure 1 of the present application. The uppermost diagram in Figure 1 shows one embodiment of a sense promoter primer that is annealed to an mRNA target nucleic acid. After synthesis of first-strand cDNA by extension of the sense promoter primer that is annealed to the mRNA template (e.g., by reverse transcription using an RNA-dependent DNA polymerase), a linear sense promoter-containing first-strand cDNA is obtained. It is important to note that this linear sense promoter-containing first-strand cDNA could not be used as a template for transcription by an RNA polymerase even if a complementary anti-sense promoter oligonucleotide is annealed to the sense promoter sequence, or even if the linear sense promoter-containing first-strand cDNA were made double-stranded by extending a primer that annealed to the 3'-end of the linear sense promoter-containing first-strand cDNA using a DNA polymerase. Incubating such a template with an RNA polymerase that binds to the double-stranded RNA polymerase promoter would not yield any product complementary to the template strand. This is because the sense promoter sequence is joined to the wrong end of the template for transcription. In order to be operable as an RNA polymerase promoter, the sense promoter sequence must, not only be double-stranded, but also must first be operably joined to the 3'-end of the template strand sequence that is transcribed. Therefore, if a complementary anti-sense promoter oligonucleotide is annealed to the sense promoter sequence of the linear sense promoter-containing first-strand cDNA, an RNA polymerase that recognizes the promoter will bind to the resulting double-stranded promoter, but it will not be able to transcribe the first-strand cDNA template because it is joined to the 5'-end of that template rather than to its 3'-end. Thus, one important aspect of the claimed method is the step of covalently joining the sense promoter sequence in the linear sense promoter-containing first-strand cDNA to the 3'-end of the template strand by ligating its 5'-end to its 3'-end, as shown in the next step in Figure 1. Thus, I believe the use of the ligation step to operably join the sense promoter sequence to the 3'-end of the primer extension product is also a novel and non-obvious aspect of the claimed method. I believe that, prior to the present application, there was a failure of others to recognize this method for operably joining the sense promoter sequence to the 3'-end of the primer extension product so that it would serve as the template for transcription. I believe this also explains why only anti-sense promoter primers had been used to amplify target nucleic acid sequences prior to the present application. Those who developed the methods in the prior art had shown that if an

anti-sense promoter primer that is annealed to a target nucleic acid sequence is extended using a DNA polymerase and then the linear anti-sense promoter-containing first-strand cDNA is made double-stranded by extending an RNA or DNA primer that anneals to the linear anti-sense promoter-containing first-strand cDNA, the resulting second-strand cDNA will have a sense promoter sequence joined to its 3'-end. Then, the second-strand cDNA with the sense promoter sequence joined to its 3'-end in the resulting double-stranded cDNA will serve as a template for transcription by an RNA polymerase that binds the double-stranded promoter. Thus, the methods in the prior art used an anti-sense promoter primer rather than a sense promoter primer to amplify a target nucleic acid sequence. The RNA synthesized was *anti-sense with respect to the target nucleic acid sequence* to which the anti-sense promoter primer annealed. Therefore, I believe that, prior to the present application, it was not known how to use a sense promoter primer to amplify a target nucleic acid sequence to generate RNA that was sense with respect to the target nucleic acid sequence in a sample. For example, it was not known how to use a sense promoter primer to generate transcripts that exhibit a nucleic acid sequences which are identical to one or multiple target RNA molecules in a sample, such as all of the mRNA molecules in a sample.

9. I believe that the description in paragraph 8 above also explains why anti-sense promoter primers, which exhibit an anti-sense promoter sequence in their 5'-portion, were well known in the prior art, whereas sense promoter primers were not known. I note that anti-sense promoter primers were used in many of the prior art methods for amplifying target nucleic acid sequences, such as in those methods listed, referenced or described in paragraphs [0007], [0008], and [0011] of the present application. Examples of methods in the prior art which use anti-sense promoter oligos include continuous amplification reaction (CAR), nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA), 3SR, and the so-called "Eberwine aRNA amplification method."

10. Various DNA cloning vectors or other DNA molecules are known in the art which contain RNA polymerase promoters, such as T7, T3, or SP6 RNA polymerase promoters. For example, a number of plasmid, fosmid, phagemid, Lafmid, and other cloning vectors that contain one or two RNA polymerase promoters are well-known in the art. Vectors such as

Pharmacia's pT7T3 phagemid vector and Promega's Riboprobe Gemini vectors were used by Soares et al. in U.S. Patent No. 5,830,662, which reference was cited by the Examiner in the pending Office Action. However, I believe that those with knowledge in the art will know and understand that such vectors are not sense promoter primers or even anti-sense promoter primers according to the definitions in the present application. In fact, when such vectors are incubated with a ligase in order to ligate the vector to a nucleic acid, those vectors are not used as primers and cannot be defined as primers according to the definitions in the present application or even according to the definitions of a primer generally known in the art. Therefore, since Soares et al. simply ligated DNA into the vectors, I do not believe Soares et al. is relevant prior art.

11. I believe, and I believe that those with knowledge in the art would understand, that U.S. Patent No. 6,136,535 to Lorincz et al. did not use a sense promoter primer as defined in the present application. All of the promoter primer oligonucleotides described for use in the method of Lorincz et al. exhibit *anti-sense promoter sequences*. This is illustrated by the fact that all of the SEQ IDs of oligonucleotides given in the specification of Lorincz et al. exhibit anti-sense promoter sequences, as well as by careful reading of the description and careful inspection of the steps depicted in the figures in the specification of Lorincz et al. In some embodiments of the methods of Lorincz et al., the promoter primer oligonucleotides that exhibit an anti-sense promoter sequence serve as a template for generating a double-stranded promoter, but none of the steps of the presently claimed methods were used in the methods of Lorincz et al., and I believe that the methods of Lorincz et al. are not similar to, or suggestive of, the presently claimed methods.

12. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: 21 December, 2009

By: Gary A. Dahl
Gary A. Dahl, Ph.D.